Review on the extraction methods used in medicinal plants

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Abstract-

Use of medicinal plant is nowadays supported with good scientific evidences. Medicinal plants are gaining interest due to their usefulness in treatment of common diseases like cold, fever etc. The study of useful medicinal plants started with extraction techniques that contribute to extraction outcomes (yield and phytochemical content) many different ways for the extraction are known these days which can be employed for the purpose. This review focuses on the describing and comparing different extraction techniques on the basis of their working principle, efficiency and their disadvantages and hence analysing the feasibility and the accuracy of these methods.

Introduction

Medicinal plants are significant for their excellent contribution for being great source of medicinal phytochemicals that may lead to production of many useful drugs. Many phytochemicals like flavonoids and phenolics are known to have a good impact on health and cancer prevention. Plant based diet in Okinawan people has shown to extend the life span of these people so modern Mediterranean people and DASH (dietary approaches to stop hypertension) incorporated phytochemicals in diet from vegetable and plant sources. Use of phytochemicals in development and formation of cosmetics instead of harmful synthetic products has gained interest that lead to increase in research and industrial applications of plants that are useful in medicinal industry. Plants containing high ratio of flavonoids and phenolics are being studied due to their antioxidant properties that has a major role in prevention of diseases that are related to age which are caused by imbalance of oxidative products . As consequences of finding phytochemicals beneficial, and more interest in natural products in pharmaceutical and cosmetic industry, the research on these medicinal plants have become as important as research on the other conventional drugs.

The study on medicinal plants begins with the pre extraction procedures followed by extraction process which are extremely important steps in processing of useful bioactive chemicals from plant parts. Conventional methods like soxhlet extraction and maceration are more commonly used at small scale or (SME) level. Advance extraction methods are nowadays used in processing of medicinal plants such advance extraction methods are- microwave assisted extraction (MAE), ultrasound assisted extraction (UAE), and supercritical fluid extraction (SFE) etc., in which above mentioned advance techniques are used to produce higher yield at lesser cost. However, modifications to these techniques are made continuously to get still better results of extractions. Punctilious evaluation has to be made for the proper selection of extraction methods from such wide variety of methods.

This review describes different types of extraction methods with their principle, efficiency or strength, and limitation of these methods that helps in proper selection of most suitable methods.

Pre- extraction preparation of plant samples

The very first step in studying the medicinal plants is the preparation of plant sample to preserve the phytochemicals of the plant cells before an extraction. Plant parts such as leaves, fruits, barks, flowers, stem, roots etc can serve as sample and can be extracted from fresh plucked plant parts or dried parts. Pre preparation of plant sample also includes grinding, drying etc. which also influences the preservation of bio molecules in final extract.

Fresh *or* Dried sample

In studies of medicinal plants, both fresh and dried samples are used. However, dried samples are preferred over fresh sample due to the time required for the experimental design. Fresh samples are delicate and tend to degrade quicker than the dried samples, so Suleiman et al limit the time interval between experimental work and output at maximum time period of 3hours to keep the sample fresh. For example, in case of *Moringa oliefera* leaves comparison between fresh and dried sample showed that there were no significant effects in total phenolics content but there was higher content of flavonoids in air dried samples(9).

Grinded *or* Powdered sample

The powdered samples have more homogenized and smaller particles as compared to coarse particles of grinded sample. As we decrease the size of particles the surface contact between sample particles and solvent increases. Therefore the powdered particles have more surface contact with the extracting solvent. This is practically very important for the particles of sample to be in contact with extracting sample for an efficient extraction method. The particle size should be less than 0.5 mm for an ideal extraction. Suleiman et al mentioned this particle size, they prepared the particle sample at size 0.4mm in vegetable samples. Many methods are used to reduce the particle size (to make powder of the sample), these are ordinary methods like mortar and pestle, electric blenders and mills etc. Analysis of nano sized particles of *Cantella asiatica* formed by planetary ball milling (PBM) showed 83.09% higher yield as compared to micro sized particles using maceration technique for 3 days in 90% methanol(4). Size of particles also plays very important role in affecting the yield in case of enzyme assisted extraction. Using enzymes like pectinolytic and other cell wall degrading enzymes in sample preparation has a major impact of particle size as more smaller particle size increases enzyme action to great extent.

Sample drying

Drying of samples can take place in many different ways such as air drying, freeze drying (lyophilisation) and oven drying.

- **Air drying**

Air drying time may vary depending upon the type of plant part samples (e.g. leaves, stem or root) that needs to be dried, this may take from 4-8 days to months or may take a year. The plant sample was tied and hanged for exposing the plant to air at room temperature. Heat labile compounds are preserved as the sample is not force dried at higher temperature. The use of air drying is limited because this method is time consuming in comparison to freeze drying or oven drying and also this method may lead to contamination.

- **Microwave drying**

Other method is microwave drying. This method employs the electromagnetic radiations which possess both electric field component and magnetic field component in microwave region. Heating is caused by electric field through dipolar rotation, alignment on the electric field of molecules having a permanent dipole or induced dipole moment, it produces oscillations in the molecule. As a result of oscillations, collisions between molecules that lead into faster heating of the sample molecules simultaneously. This method has advantage of faster heating but this method can sometimes cause the degradation of phytochemicals of medicinal plant.

- **Oven drying**

Oven drying is another method of sample drying in pre extraction procedures. This method uses the heat energy to remove the excess moisture from the sample. This method can preserve the phytochemicals and it is the most easy and rapid method for drying of the samples. Oven drying at 45°C for 4.15 hours using 80% methanol as solvent resulted in highest antioxidant action in species *Cosmos caudatus.* Shorter time is required for the extraction method using this drying technique .But there was no effect of drying on the antioxidant effect of species *Orthsiphon stamineus* however the bioactive phytochemicals like sinensetin and rosmarinic acid content were affected by the drying method suggesting that compound is temperature sensitive. Use of oven drying is restricted for the soft and heat sensitive plant materials of high value.

- **Freeze drying**

Freeze drying method is based on the sublimation principle***(process of conversion of solid phase into gaseous phase without converting into liquid phase)***. Any liquid present in sample such as any solvent or moisture is solidified by freezing the sample at -80°C to -20°C before performing lyophilisation. After freezing the sample overnight for 12 hours the sample is immediately lyophilised to avoid the melting of frozen liquid in sample. The container containing the sample (usually test tube) is covered with parafilm poked with a needle to avoid the loss of sample during process. Freeze drying resulted into highest yield of phenolics as compared to other methods as the constituents are preserved well during this method, there is no degrading of the bio molecules of sample. However use of this method is restricted due to its high cost and its complex nature as compared to other methods of drying like oven drying, and air drying etc.

Extraction methods

Extraction is a process to separate a desired substance when it is mixed with others. The sample is dissolved in a solvent in which the compound of interest is soluble, but the other substances present are insoluble. The intial crude extracts extracted using these methods contain very complex mixture of plant metabolites, for example alkaloids, glycosides, phenolics, terpenoids and flavonoids etc. Some of the initially obtained extracts may be used as medicinal agents in the form of tinctures and fluid extracts while some of them need further processing. Several of the commonly used extraction methods are given below-

Maceration, infusion, percolation and decoction: Maceration is a method used in wine production and this method is used widely nowadays in medicinal plants research. Maceration involves soaking plant materials either powder or coarse particles in a closed mouth container with a suitable solvent and it is kept at room temperature for a period of minimum 3 days with frequent heating. The soaked sample gets soft and the cell walls of plant sample breaks that releases the soluble phytochemicals. After 3 days, the mixture is pressed or filtered. It is a traditional method, in which heat is transferred through convection and conduction. The type of compound extracted from the samples depends purely on the solvent employed for this method. Infusion and decoction both works on the same basic principle as maceration; the sample is soaked in cool or boiling water. However, the maceration period for infusion is shorter than maceration and in decoction the sample boiled in specific volume of water (e.g. 1:3 or 1:15) for a definite time. Decoction is applicable only for extraction of compounds which are stable at high temperature , hard plant parts (e.g. roots and barks). It usually gives compounds which are more soluble in oil in comparison to maceration and infusion. Another method that has similar working principle is percolation that employs a unique equipment known as percolator. Dried powdered sample is loaded in the percolator, and boiling water is added to it and macerated for 2 hours.

Strength and limitation: This extraction method is the easiest one and simplest method one can employ for extraction. However, large volume of solvents is used and organic waste is generated so proper management of the waste is needed. The volume of solvent can be reduced by introducing alteration in temperature and by choosing more suitable solvent system when such changes are not objectionable. By boiling species *Centella asiatica* at 90° C phenolics content and its antioxidant action could be increased , but with increase in extraction time the PH of the solution changed. In this method, solvents employed for the extraction purposes plays a major role in extraction of the desired compound.

Soxhlet extraction or hot continuous extraction

In this method, finely powdered sample is used which is packed in a “thimble” made from a strong filter paper or cellulose or a porous bag is often used instead, which is placed, in chamber of the Soxhlet apparatus. Extraction solvents are heated in the RBF, which gets vaporized and moves into the sample thimble, there it condenses in the condenser and solvent then drops back. When the solvent in soxhlet apparatus reaches the arm, the liquid

contents empties into the RBF again and this process is repeated again and again until the extraction is completely done.

Strength and limitation: This method employs a lesser quantity of solvent in comparison to maceration. However, the Soxhlet extraction has a disadvantage. There are chances that worker may get exposed to dangerous and organic solvents that catches fire easily, and emissionsof toxic substances can take place while performing this type of extraction. Solvents needed for the extraction should be highly pure however this might lead to increase in expenses. This technique may cause environmental pollution problem as it is not so environmental friendly technique this is not so in case of advance extraction methods such as supercritical fluid extraction (SFE). The sample should be ideally dried and finely powdered sample for Soxhlet extraction and many more factors need to be considered for this method are- temperature, solvent-sample ratio and agitation speed.

Microwave assisted extraction (MAE)

MAE employs microwave radiations to aid migration of phytochemicals from the sample matrix into the solvent. Microwave energy causes heating near the surface of the materials by the interaction of dipoles of polar and polarisable materials (e.g. solvents and sample) and heat is then transferred by conduction. Dipole rotation of the molecules induced by microwave electromagnetic radiations damages the hydrogen bonding. This enhances the movement of dissolved ions and leads to solvent penetration into the matrix. In non-polar solvents, heating does not to sufficient extent as the energy is transferred by dielectric absorption only. MAE can be considered as selective method that is used for polar molecules and solvents with high dielectric constant only(1).

Strength and limitation: This technique utilizes smaller solvent volume and reduces extraction time as compared to traditional methods such as maceration and Soxhlet extraction. This method has limitations as this method is only used for the samples which are stable under microwave radiations such as phenolic acids (gallic acid and ellagic acid), quacertin, isoflavin and trans-resveratro. Additional cycles of MAE (e.g. from 2 × 10 s to 3 × 10 s) lead to rapid decrease in the yield of phenolics and flavanones, mainly due to the oxidation of compounds. Tannins and anthocyanins may not be suitable for MAE as they degrade at high temperature.

Ultrasound-assisted extraction (UAE) or sonication extraction UAE

It utilizes ultrasound of range from 20 kHz to 2000 kHz. The ultrasound produces cavitations which enhances the surface contact between solvents and samples and increases permeability of cell walls. Physical and chemical properties of the materials when subjected to ultrasound are varied and the plant cell wall breaks; releasing the matrix of cell and facilitating the mass migration of the solvents into the plant cells. This technique is easy and less costly and this technology can be used in both small and larger scale extraction of phytochemical.

Strength and limitation: The advantage of UAE is the reduction in extraction time and less solvent consumption. However, use of ultrasound energy more than 20 kHz may affect the active phytochemicals through the formation of free radicals.

Other extraction methods

Other methods such as **accelerated solvent extraction (ASE)** and **supercritical fluid extraction (SFE)** are also used in the extraction of plant materials. These methods are not much popular because these are expensive techniques, however the efficiency of these methods is good.

Accelerated solvent extraction (ASE)

ASE is an efficient form of liquid solvent extraction as compared to conventional methods such as maceration and Soxhlet extraction, as this method use lesser amount of solvent. Sample is loaded alongwith inert material such as sand in the stainless steel extraction cell. This is to prevent sample from forming a clump and blocking the system tubing. Layers of sand-sample mixture are packed in cell, in between cellulose filter paper and the sand layers. The temperature and pressure can be varied for each individual sample in this automated technique and reduces the extraction time, requires less than an hour for extraction(8).

Supercritical fluid extraction (SFE)

Supercritical fluid (SF) or also referred as dense-gas is a substance that has the physical properties of both liquid and gas phase at its critical point. SF has more gas like behaviour but has the solvating property of a liquid. An example of SF is CO_2. It behaves as SF at above 31.1°C and 7380 kPa. Supercritical CO_2 is useful in plant extraction due to its property of behaving as excellent solvent for non polar analytes. CO_2 is less costly and easily available and has low toxicity. Polar compounds can also be extracted using SC-CO_2 by adding some amount of ethanol or methanol. As SC-CO_2 vaporizes at room temperature, the extracted compound can be purified easily.

Discussion

All the extraction techniques employ different solvents in the procedures therefore yield is critically influenced by the solvent system. However, there is no effect of the solvent volume used during three methods that are maceration, MAE and UAE on the biological activity. Maceration as compared to other methods have been suggested by some researchers to be a more useful, convenient method for small and medium enterprises (SMEs). However, a major issue in maceration method is the issue of organic waste as compared to MAE and UAE, which are sometimes also known as the “Green methods”. However, the crude extract in these methods contain other metabolites, this particular fact suggests that further isolation and purification of extracts are needed, which is further more complex process and time consuming also. This is not necessary if preparation and extraction are done properly. Most Suitable and optimum conditions for each extraction methods also plays vital role in

extraction. Several factors such as temperature, heat and light needs to be analysed carefully to extract compounds that are thermo labile. Solvent sample ratio have no significance effect on the yield, suggesting that unnecessary large volume of solvents can be avoided. Each plant has unique optimal conditions for efficient extraction. All the influential factors such as temperature, solvents, agitation speed and etc. might have the ability to enhance extraction, but without proper judgment may lead to degradation of compounds. However, when purity is taken into consideration, advanced extraction technology such as ASE should be employed.

Conclusion- Both the pre-extraction and extraction stages in extraction are equally important in the study of medicinal plants. The sample preparations such as grinding and drying have major impact on the efficiency and phytochemical constituents of the final extractions; that in turn have an effect on the final extracts. It can be concluded that, no extraction method is the ideal method; it greatly depends upon the plant material.

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